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# NOTES ON THE GENUS APHANOMYCES, WITH A DESCRIPTION OF A NEW SEMIPARASITIC SPECIES

By J. N. COUCH

PLATES 26–33

The genus *Aphanomyces* should be of particular interest to students of fungi and of parasitism in general because it shows in its species such a remarkable range in growth habits. This range extends from the nearly purely saprophytic species as *Aphanomyces laevis*, through the condition of obligate parasitism as found in *A. parasiticus* on species of *Achlya*, to the obligate parasitism of *Aphanomyces phycophilus* on conjugate algae. Quite recently Drechsler (Jones and Drechsler, '25) has described a new species of *Aphanomyces*, *A. euteiches*, which parasitizes the roots of peas but is culturable as a saprophyte.

In the present paper some general remarks on the growth habits of the members of the genus are given, together with notes on *A. phycophilus*, notes on a new form of *A. laevis*, and a description of a new species which shows a remarkable range in its growth habits. This work was done in the botanical laboratory of the University of North Carolina under the direction of Dr. W. C. Coker, to whom the writer is greatly indebted for criticism and advice. The writer also wishes to thank Miss Alma Holland for inking in plate 32 and part of plate 33.

The genus *Aphanomyces* as at present constituted can be divided into the following groups according to the saprophytic or parasitic habit of the species, as is shown in table I.

None of the species is an obligate saprophyte, as the three species, A. *laevis*, *A. scaber*, and *A. stellatus*, which are almost invariably saprophytic in their habit, have all been found growing parasitically on algae or within the threads of other water molds. Coker ('23) described a strain of *A. laevis* parasitizing desmids and diatoms, while the present writer has found *A. scaber* and *A. stellatus* growing within the living threads of species of *Achlya*. The new species herewith described may be cultured as a pure saprophyte or may be cultured as a parasite and saprophyte at the same time. Two of the species, *A. phycophilus* and

*A. parasiticus*, are apparently obligate parasites, and all efforts to culture them saprophytically have failed.

TABLE I

OBLIGATE SAPROPHYTES:

None

HEMI-SAPROPHYTES:

*A. laevis* (Rarely parasitizes diatoms and desmids; oogonia smooth, 18–33$\mu$ thick; with antheridia)

A form (With a few spiny oogonia and rarely with oogonia containing 2 eggs)

*A. scaber* (Rarely on *Achlya;* oogonia 15–24$\mu$ thick, wall uneven to tuberculate; antheridia absent in Chapel Hill form)

*A. stellatus* (Rarely on *Achlya;* oogonia 22–23$\mu$ thick, wall covered with blunt papillae; antheridia present on all oogonia)

*A. exoparasiticus* (Parasitizes all Phycomycetes tested; oogonia 25–40$\mu$ thick; egg 24–29$\mu$ thick)

*A. euteiches* Drechsler (Parasitizes the roots of peas; culturable as a saprophyte on corn meal agar, etc.; oogonia 25–35$\mu$ thick, inner surface of oogonial wall sinuous, eggs 18–25$\mu$ thick)

OBLIGATE PARASITES:

*A. phycophilus* (On conjugate algae; oogonia 40–50$\mu$ thick, spiny; egg 36$\mu$ thick)

*A. parasiticus* (On *Achlya;* oogonia 14–22$\mu$ thick; egg 12.8–21.7$\mu$ thick; threads running inside the host except in reproduction)

**Aphanomyces phycophilus** de Bary

Threads larger than in other species of the genus, 7.4–14$\mu$ thick; growing longitudinally through the cells of *Spirogyra* and developing numerous, considerably branched haustoria which intermingle with the chromatophores, causing their disorganization and collapse into irregular masses. Threads often extending outside the host. Asexual reproduction not observed. Oogonia formed in considerable abundance, usually extramatrical but rarely also formed within the host threads; borne on lateral stalks equal in length or slightly longer than the diameter of oogonia; oogonia 40–50$\mu$ in diameter counting the numerous, conspicuous, blunt spines which are 6–9.3$\mu$ long; wall becoming dark brown upon maturity. Egg single in the oogonium, 30–38$\mu$ thick. Antheridia of diclinous or androgynous origin, usually one on an oogonium.

This species can readily be distinguished from other members of the genus by its thicker threads, parasitic habits, and large bluntly spiny oogonia, and by the absence or extreme rarity of asexual reproduction.

This interesting parasite has heretofore been reported from Germany

by de Bary and from Michigan by Kauffman, and from Indiana by Weatherwax. Last spring (1924) in some algal material collected from near Georgetown, S. C., we found an abundant lot of the fungus parasitizing the threads of *Spirogyra;* this spring (1925) we collected it several times on *Spirogyra areolata* and an undetermined species of *Spirogyra*. Since de Bary, who first described the fungus, has made the only attempts to culture it, but was unable to induce the plant to grow, it seemed a matter of interest to make a rather exhaustive effort to culture the fungus on artificial media and especially on different kinds of nutrient agar.

Healthy threads of the fungus, some of which were extending out of the *Spirogyra* threads, were cut off, some with and others without parts of the *Spirogyra*, and were placed on corn- and oat-meal agar plates. Healthy threads of the fungus were also placed on bits of boiled corn grain, hemp seed, termite ants, mushroom grubs, etc. All efforts, however, to culture the fungus were unsuccessful.

As no one has ever observed with certainty any form of asexual reproduction in the plant, an effort was made to induce the formation of sporangia by transferring filaments of the *Spirogyra* containing numerous healthy threads of the parasite to fresh, pure water, a process which is usually successful in inducing the formation of sporangia in other species, but without any success in the case of the present plant.

**Aphanomyces laevis, a form**

Threads delicate, averaging about 6$\mu$ thick, straight or slightly sinuous, not rarely branched. Sporangia of the same thickness as the threads, very long. Spores clustering at the sporangial tip upon discharge, diplanetic, 8–11$\mu$ thick. Oogonia usually borne on much curled, racemose branches, but not rarely on short, straight branches; often in dense clusters with 20 or more oogonia in a cluster, varying from 18–48$\mu$ in diameter, most 29–35$\mu$ thick, spherical or slightly subspherical, wall smooth except in old cultures, and then the oogonial walls often appear spiny, due to the disappearance of part of the antheridial walls and the persistence of the other parts, often yellow in old cultures, apparently unpitted. Antheridia on all oogonia, one, two, or several, not rarely nearly covering the oogonium; antheridial tubes developed which apparently penetrate the egg. Eggs single in the oogonium, very rarely two may be present, not completely filling the oogonium, 14–35$\mu$ thick, most between 22–28$\mu$ thick, structure as in *A. laevis*. Recognized twice in over a hundred collections around Madison, Wisconsin, and then from Lake Mendota opposite stock pavilion, No. 31, July 1, 1923, and No. 48, July 20, 1923.

This plant differs from the form of *A. laevis* treated by Coker ('23) in the larger oogonia and eggs and the brown color of the former and also by the oogonia in this variety occasionally appearing spiny due to the disintegration of part of the antheridial wall. Very rarely oogonia may be observed with two eggs, a condition heretofore not reported in the genus. The oogonia in this form quite often show a tendency to form in dense clusters with often as many as twenty or more oogonia in a cluster. Such clusters can easily be recognized with the unaided eye and give the cultures a very distinctive appearance.

Petersen ('10) suggested that this species occurs in many varieties and this certainly seems to be the case. De Bary originally described the eggs as 27–33$\mu$ thick, and most of the eggs of Coker's plant described from Chapel Hill are 19–22$\mu$ thick. Coker ('23) has described also a variety parasitic on algae.

**Aphanomyces exoparasiticus** Coker and Couch n. sp.

Mycelium facultatively a semi-parasite on the threads of other Phycomycetes; also culturable as a pure saprophyte on corn meal agar seeds, and insects (mushroom grubs, ant larvae, etc.). Hyphae, when growing as a semi-parasite, twining about the threads of the host and rapidly destroying them; when growing as a saprophyte on vegetable or animal matter in water, much as in *A. laevis* or *stellatus*, but not so strongly developed; on corn meal agar the mycelium is abundantly developed forming rhizoid-like, lateral outgrowths much like those entwining the host threads when the plant is growing semi-parasitically. Sporangia formed in abundance on hemp seed, rather sparingly developed when growing as a semi-parasite in water, not developed in solid media; indistinguishable from those of *A. laevis* or *A. scaber*. Spores 8.1–11.1$\mu$, averaging about 9.3$\mu$ thick, diplanetic. Sexual organs abundantly developed under most conditions, very numerous when growing saprophytically on corn meal agar, fairly abundant when growing semi-parasitically on other fungi in water, rarely or not at all developed when growing saprophytically on bits of hemp seed or corn grains in water. Oogonia usually borne on lateral stalks which are several times longer than the diameter of the oogonia; 28–40$\mu$ thick, most 33–37$\mu$ thick, not counting the long, usually sharp pointed, rarely short and blunt, and very conspicuous spines which vary from 5.5–14$\mu$ long; spines 10–20 to an oogonium; oogonial wall 1.8–2.5$\mu$ thick. Eggs single in the oogonium, nearly filling the latter, 24–30$\mu$ thick, spherical, rather thick-walled, 2–2.5$\mu$ thick, with a centric or subcentric, large oil drop surrounded by granular protoplasm. Antheridial branches diclinous, arising usually from a thread near the base of the oogonial stalk and twining around the latter; antheridia either short and tuber-like or often long and wrapping around the oogonia, coming in contact with

the oogonia very early, completely emptying their contents; the empty antheridia thick-walled and long persistent.

This peculiar plant was first observed growing as a semi-parasite on an unidentified species of *Pythium*, collected by Mr. J. V. Harvey, in the early spring of 1924.

In its vegetative growth and asexual reproduction as observed when the plant was growing saprophytically on bits of seeds in water, it could hardly be distinguished from other saprophytic species of the genus. Its peculiar sexual organs, however, which are developed under nearly all conditions of culture serve to readily distinguish the plant from all other members of the genus. From the two plants to which it seems closest, *A. phycophilus* and *A. parasiticus*, it can easily be distinguished by the larger oogonia and eggs of the former, and the smaller oogonia and eggs of the latter, and also by the fact that the first mentioned plant is an obligate parasite on *Spirogyra*, while the second mentioned one is an obligate parasite within the threads of *Achlya*.

The mycelium of this peculiar plant when growing saprophytically on bits of boiled corn grain, hemp seed, etc., or on ant larvae or grubs in water presents much the same appearance as does that of other saprophytic members of the genus. On corn meal agar made up according to our standardized formula (4 heaping teaspoons corn meal in 1 liter of water heated over water bath 1 hour. Filter. To filtrate add 12 grams agar. Autoclave 15–30 minutes 10–15 lbs. pressure), the fungus grows rather slowly as compared with most other water molds, growing at about the rate of 0.5–0.7 cm. every twenty-four hours, while *S. ferax*, to give an example of a rapid grower, grows at least 2 cms. every twenty-four hours. The mycelium on agar while not so dense as in other saprophytic species in the genus is made up of thicker, more sinuous, and more branched threads. When growing saprophytically on agar, there quite often grow out from the main threads numerous, short, much branched, rhizoid-like threads much like those entwining the host threads when the plant is growing semi-parasitically.

When growing in water on bits of vegetable or animal material in company with another water mold as a *Pythium*, or an *Achlya*, the *Aphanomyces* hyphae develop numerous branched, rhizoidal attachments which fix themselves to the hyphae of the host. These rhizoidal branches, which may be regarded as superficial haustoria, twine around the threads of the host, establishing a very intimate communication with them. Since the material was not studied by sectioning and staining, it is impossible to say just what were the relations between the protoplasm

of the parasite and that of the host. A painstaking search was made on living material with a Leitz binocular and an apochromatic oil immersion objective to find cases in which the walls between host and parasite were dissolved. Only a few clear cases could be found, one of which is shown on plate 33, fig. 10. Here, at the letter A, the host wall seemed to have disappeared entirely, thus permitting a direct communication between the protoplasm of the parasite and host. In no case could any interchange of protoplasmic material be observed. The effect upon the host, however, is very marked and rapid. The protoplasm of the host threads in infected regions soon becomes disorganized, presenting a coarsely irregular, granular, vacuolate appearance. The host thread, apparently in order to protect itself, establishes a cross wall between the healthy and unhealthy regions. Meanwhile the haustoria multiply until in many cases they completely cover the host thread for a considerable distance. The parasitized regions in the host threads finally present a glistening look as though either empty except for sap or filled with oil. The osmic acid test failed several times to give an oil reaction, while Congo red gave a decided reaction for cellulose in the parasitized region.

The mycelium when growing in water forms numerous sporangia which may begin discharging their spores within twenty-four hours after the substratum was inoculated. The development of the sporangia, the discharge of the spores and their subsequent history parallels that in the other commoner species.

The oogonia are formed under most conditions of culture. They are developed in greatest abundance when the plant is growing on corn meal agar in the presence of a congenial host, as *Pythiopsis cymosa*. Many are formed when grown as a pure saprophyte on corn meal agar. A fair number are formed when growing on bits of corn grains in water in the presence of another watermold, while only a few are formed on purely saprophytic cultures in water.

When growing on agar, the oogonia are formed in daily concentric circles, the first circle appearing after about two days about 1.5 cms. from the point of inoculation. The width of the circle when the fungus was growing on our standard corn meal agar, with the temperature about 22°C., in company with *Pythiopsis cymosa* was about 0.4–0.5 cm.

This regular daily appearance of a new circle of oogonia offers a parallel case to the daily appearance of a circle of sporangia in *Thraustotheca primoachlya*, and *Achlya orion* and is of particular interest as it complements the work of Smith ('21) on *Saprolegnia diclina*, in which he

found that by far the greater part of the nuclei divided during the latter part of the night to form the spores on the following day.

## EXPERIMENTS TO DETERMINE THE RANGE OF PARASITISM

Numerous experiments have been made to determine the range of the growth habits of the plant. The fungus as has been mentioned above was first found growing with and parasitizing many of the threads of an unidentified species of *Pythium* in water on a piece of corn grain. Single spore cultures were made by isolating a cluster of the *Aphanomyces* spores and placing the cluster on a corn meal agar plate in a drop of water. About ten hours later many of the spores had emerged from their cysts, and sprouted into minute germ tubes. Several of these sprouting spores were cut out and separately planted on fresh corn meal agar plates. A strain descended from a single spore culture has been used in making the preceding descriptive notes and the following experiments.

Several attempts have been made to induce the plant to grow as a pure parasite on the threads of other molds in water. All efforts however in this line so far have been unsuccessful. The probable explanation is that the parasite grows very poorly in water, while all the other species tested grow very luxuriantly in water. The tests were made as follows: young cultures of the prospective host and parasite growing on bits of boiled corn or hemp seed were put in the same Petri dish so that their threads were in contact. It was anticipated that the parasite would attack the prospective host threads and then that the connections between the parasitized area and the saprophytic substratum might be severed by cutting the *Aphanomyces* threads, after which, if the *Aphanomyces* were capable of living as a pure parasite, it would continue to grow receiving its food entirely from the host. The results of these experiments may be summarized as follows:

1. *Aphanomyces* × *Saprolegnia ferax*, young culture. No parasitism, the *Saprolegnia* overgrew the *Aphanomyces* substratum.
2. Experiment repeated with same results.
3. Experiment repeated, except a mature culture of the *Saprolegnia* was used; results as above.
4. *Aphanomyces* × *Achlya* (sp.), young culture. No parasitism, *Achlya* overgrew the *Aphanomyces* substratum.
5. Experiment repeated, except an old culture of the *Achlya* was used, with same results.
6. *Aphanomyces* × *Dictyuchus monosporus*, oogonial strain A. No parasitism. *Dictyuchus* overgrew *Aphanomyces* substratum.

7. *Aphanomyces* × *Dictyuchus monosporus*, antheridial strain. No parasitism. *Dictyuchus* overgrew *Aphanomyces* substratum.
8. *Aphanomyces* × *Pythium* sp. *Pythium* threads slightly parasitized only where the threads of the two plants were placed in contact. *Aphanomyces* did not spread over the *Pythium* culture.

Since the *Aphanomyces* would not grow well in water it seemed advisable in testing the range of the plant's parasitic habit to grow it on corn meal agar plates, a substratum on which the fungus grows well. The tests were made by inoculating a corn meal agar plate with threads from a pure culture of the parasite and also pure threads of the prospective host. The two fungi were usually placed about 2 cms. apart. Phycomycetes, Ascomycetes, and one Basidiomycete have been used in making the tests.

Several types of reactions have been obtained which may be summarized as follows:

1. Host inhibited, the parasite not inhibited, the latter forming a perfect circle of growth. Host threads parasitized.
2. Host plant considerably or entirely inhibited, the parasite more or less inhibited. Host threads parasitized.
3. Mutual inhibition, parasitism.
4. Prospective host at first inhibited and parasitized but later overgrowing the *Aphanomyces*.
5. Mutual inhibition, no parasitism.
6. Prospective host not inhibited, the *Aphanomyces* inhibited and unhealthy in region next to the prospective host. No intermingling of threads.
7. Type of reaction depending upon temperature.

1. Host inhibited and much parasitized, parasite not inhibited, the latter forming a perfect circle of growth (see pl. 28, fig. 1). All members of the *Saprolegniaceae* tested gave this general type of reaction, including *Pythiopsis cymosa, Achlya orion, A. megasperma, Thraustotheca clavata, T. primoachlya,* and *Dictyuchus monosporus* (both oogonial and antheridial strains). Most of the above species are rather slow growers on corn meal agar, growing as slowly as or even more slowly than the *Aphanomyces*. In the case of *Saprolegnia ferax,* however, which grows very rapidly, the *Aphanomyces* parasitizes it and inhibits its growth somewhat but the threads of the latter turn aside and grow around the parasite, finally completely overrunning it.

All members of the genus *Pythium* tested gave the same general type of reaction as that described above for *Pythiopsis cymosa, Achlya orion,* etc. The photograph (pl. 26) shows a plate of *Aphanomyces* and

*Pythium* sp. in which the *Pythium* is parasitized and its growth inhibited while the parasite continues its growth.

Using this species of *Pythium* (No. 53) as a host, some experiments were carried out to see if immunity was acquired by the host after it had been parasitized. Host threads out on the margin of the contact zone which were parasitized in a region three or four millimeters back from the tips were cut out in agar squares (fig. 4) and placed on a corn meal agar plate at the same time with a square of agar containing the *Aphanomyces* threads (fig. 5). The *Pythium* below and on the sides was not parasitized and considerably overgrew the *Aphanomyces*, in the middle above (fig. 5). Agar squares containing *Pythium* threads from this non-parasitized growth were now cut out and put on a plate with the *Aphanomyces* and in this experiment the *Pythium* was parasitized as formerly. This series of experiments was repeated with the same results. It seems, therefore, that a temporary immunity may be acquired by the *Pythium*.

2. Host plant considerably or entirely inhibited, the parasite more or less inhibited. Host threads parasitized. This type of reaction was obtained in crosses with species of *Mucor* as *Mucor genevensis* and *M. Vuillemini*. Both of these species grow at about the same rate on corn meal agar as the parasite, and therefore when one of these Mucors was planted with the parasite on opposite sides of the dish, their threads would meet after about two days in the center of the dish. When the threads of the host and the parasite touched, the host threads stopped growing; the parasite, however, though much slowed down, continued its growth for some distance among the host threads, parasitizing them. Reactions with *Mucor parasiticus* (*Parasitella simplex*) also belong in this type. This latter species grows, as a rule, rather slowly as a saprophyte on corn meal agar, the intramatrical hyphae being rather sparingly developed. The *Aphanomyces* when plated with this plant continued its growth within the agar, parasitizing the intramatrical threads of the mucor. The growth of the mucor was inhibited.,

Reactions with *Rhizopus nigricans* might also be put in this type, but the *Rhizopus*, when its intramatrical hyphae were impeded and parasitized by the *Aphanomyces*, resorted to the method of spreading over its substratum by stoloniferous growth, and spread slowly over the *Aphanomyces* culture. Wherever the *Rhizopus* threads or runners came in contact with the agar and sent down rhizoids among the *Aphanomyces* threads, the latter quickly parasitized the *Rhizopus*.

In a culture of *Rhizopus* with *Aphanomyces* some observations were

made on the early stages of infection. The two fungi planted on the same dish May 5 were just coming in contact May 8, the *Rhizopus* threads having grown to a length of 4 cms., the *Aphanomyces* threads 1 cm. Out on the margin where the threads of the two fungi were apparently approaching each other but were not yet in contact, a *Rhizopus* and *Aphanomyces* thread which were growing in such directions that they seemed as though they would come in contact were selected for observation. After several minutes the *Aphanomyces* thread changed its direction of growth, slightly bending away from the *Rhizopus* thread which was growing toward the *Aphanomyces* at a rapid rate. At this stage the two hyphae were sketched (plate 33, fig. 1); they were 0.07 mm. apart. Figure 2 was made five minutes later and in two minutes more the two threads were in contact (fig. 3).

In seven minutes the *Rhizopus* thread had grown 0.07 mm. not counting the bending away of the *Aphanomyces* thread. In this case only the tip of the *Rhizopus* thread touched the *Aphanomyces*, and yet within three minutes after the two threads were in contact the protoplasm of the *Rhizopus* thread for a considerable distance from the tip back was becoming very coarsely granular and showing marked signs of disorganization (fig. 4). In five minutes more the part of the *Rhizopus* thread in which the contents were disorganized was cut off from the healthy part of the thread by a cross wall (fig. 5). The parasite having killed part of the host thread now quickly sends out one or more small threads which coil about the host (fig. 6).

The above observation repeated several times in cultures of *Aphanomyces* with *Rhizopus* and the numerous other Phycomycetes parasitized showed that no lethal substance is given off which kills the host in advance but that the host threads are killed only when in contact with the parasite and then with remarkable rapidity.

3. Mutual inhibition, parasitism. Both organisms grow toward each other until they are in contact. The *Aphanomyces* parasitizes the other fungus only along the lines of contact, only a slight intermingling of threads. This type of reaction results when the parasite is plated with other species of the same genus, as *Aphanomyces laevis* (see pl. 28, fig. 2).

4. Prospective host at first parasitized but later overgrowing the *Aphanomyces*. This type of reaction was obtained when the *Aphanomyces* was cultured with *Syncephalis* sp. The *Syncephalis* grows at about the same rate as the *Aphanomyces*, the two fungi meeting about the center of the dish. In the region where the two fungi met, the

*Syncephalis* was parasitized. Its growth however was not inhibited; on the contrary it rapidly overgrew the *Aphanomyces* culture preferring the region of agar occupied by it to the region of agar as yet unoccupied by either fungus. These two fungi have been cultured together several times and a careful search was carried out for a case where the *Syncephalis* (which is a facultative parasite on *Mucor* species) was parasitizing the *Aphanomyces* but no such condition could be found.

5. Mutual inhibition, no parasitism. Both organisms grow toward each other until they are in contact or nearly in contact and then growth ceases, both organisms remaining healthy. This type of reaction is obtained when two cultures of the parasite are plated together and when the *Aphanomyces* is plated with *Sordaria* sp. (pl. 27). The *Sordaria*, which is a very rapid grower on agar, growing at about the rate of 3.5 cms. a day in summer room temperature, far outstripped the *Aphanomyces* in growth. The threads of the two fungi somewhat intermingled along the line of contact, the *Sordaria* forming a dense row of perithecia along this line. Practically the same results were obtained when the parasite was plated with *Aspergillus niger* and several species of *Penicillium* except that neither of the latter formed perithecia.

6. Prospective host not inhibited, the *Aphanomyces* inhibited and unhealthy in the region next the prospective host. No intermingling of threads. The *Aphanomyces* threads near the ends of the contact region curving considerably to avoid the prospective host and those near the center of the contact region showing a very unhealthy condition, apparently dying. This type of reaction resulted between the *Aphanomyces* and *Schizophyllum commune*. No other Basidiomycetes have been tested.

7. Type of reaction depending upon temperature. Cultures of *Aphanomyces* with *Allomyces arbuscula* belong in this type. A culture was made Sept. 14 and kept in room temperature. On Sept. 30 the culture was examined: the *Aphanomyces* had covered the agar plate while the *Allomyces* had grown very poorly, the longest threads reaching a length of 0.4 cm. The *Allomyces* was much parasitized (fig. 6).

Another inoculation was made Sept. 10 and kept in the oven with the temperature at 34°C. At this temperature the *Allomyces* grew luxuriantly while the *Aphanomyces* grew poorly. Examined and figure 7 made Sept. 18. No parasitism. The *Allomyces* threads overgrowing the *Aphanomyces* cultures, the *Allomyces*, however, being slightly slowed up in this region. The *Aphanomyces* threads showed a very peculiar

branching habit near their outer margin of growth, the individual threads branching considerably and becoming thicker and septate in places, resembling in appearance the threads of *Allomyces*.

Another culture was made Sept. 14 and kept in the oven with the temperature at 34°C. for two days. The *Aphanomyces* grew very poorly; the *Allomyces* grew to a length of about 1 cm. from the point of inoculation. The culture was removed to room temperature on Sept. 16. Examined and figure 8 made Sept. 30. The *Aphanomyces* had covered the dish; the *Allomyces* had grown very slightly after being changed to room temperature but had formed many sporangia. The *Aphanomyces* was parasitizing the *Allomyces*, attacking the sporangia usually.

Several attempts were made to induce the plant to grow as a parasite on algae. Two species of *Spirogyra* were used, one a fairly large species with 4–5 chromatophores; the other a smaller species with one chromatophore. Squares of agar containing growing, healthy threads of the fungus were cut out and placed on the *Spirogyra* threads. The *Aphanomyces* threads grew out in considerable numbers to a distance from the agar of 2–4 mm., forming numerous sporangia from which spores were discharged. These experiments were kept under observation for three weeks but no infection took place.

## Discussion

Kern ('15) discusses briefly parasites as an aid in determining genetic relationships in their hosts, citing some evidence from his own work which seems to show that parasites may be used as an aid in discovering specific and generic relationships.

Matruchot ('03) has shown that *Piptocephalis Tieghemiana* would live as a parasite on any of the Mucorales but would not parasitize any fungus from any of the other groups, numerous types of which were tested. He concluded therefore that the parasitism of *P. Tieghemiana* on any fungus showed the taxonomic relationship of that fungus to the Mucorales. On this basis with the absence of sexual reproduction, he classed *Cunninghamella africana* Matruchot (= *C. echinulata* Thaxter) with the Mucorales. Blakeslee ('05) shortly afterwards reported the zygotes of this form, thus showing conclusively that this plant belonged to the Mucorales.

In the experiments which have been described above for *Aphanomyces exoparasiticus* a similar relation has been shown to exist between host and parasite as that described by Matruchot ('03) for *Piptocephalis*

*Tieghemiana*, and it might be concluded with a reasonable degree of assurance, provided there were no structural characters to negative the reasoning, that an unknown fungus which the *Aphanomyces* would use as a host was a Phycomycete.

## SUMMARY

1. The genus *Aphanomyces* may be subdivided according to the growth habits of its species into three groups: (1) Usually saprophytic in nature but occurring also as a parasite; (2) Usually parasitic in nature but culturable as a saprophyte or as a parasite and a saprophyte at the same time; (3) obligate parasites.

2. *Aphanomyces phycophilus* is reported for the first time in the eastern United States.

3. A heretofore unreported form of *A. laevis*, distinguished by the occasional spiny oogonia and the rare occurrence of two eggs in the oogonium, is described.

4. A species of *Aphanomyces* closest to *A. parasiticus* but differing from that species in the size of the oogonia and eggs and especially in growth habits is described as new with the name *A. exoparasiticus* because of its habit of sending its haustoria around the threads when growing as a semiparasite on other molds.

5. Numerous experiments are described which have been carried out to determine the range of parasitisim of the new species. It has been found that the new species parasitizes all Phycomycetes tested, but that Ascomycetes and Basidiomycetes so far tested are immune. *Spirogyra* also is immune.

UNIVERSITY OF NORTH CAROLINA,
CHAPEL HILL, N. C.

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## EXPLANATION OF PLATES

### PLATE 28

Fig. 1. *Aphanomyces exoparasiticus* above with *Pythiopsis cymosa* below. Cultures five days old. The *Aphanomyces* had formed a perfect circle of growth and three distinct concentric circles of oogonia. The *Pythiopsis* was checked in its growth when it came in contact with the *Aphanomyces* and was much parasitized.

Fig. 2. *Aphanomyces exoparasiticus* above with *Aphanomyces laevis* below. Cultures five days old. Mutual inhibition, with a very slight intermingling of threads. Parasitism along the line of contact. The parasite with three distinct circles of oogonia, while the three denser regions of the host are formed by considerable branching of the hyphae.

### PLATE 29

Fig. 3. *Aphanomyces exoparasiticus* above with *Pythium* No. 53 below. Cultures three days old. *Aphanomyces* parasitizing the *Pythium*.

Fig. 4. Same as above but two days later. The growth of both plants inhibited, the *Pythium* much more than the *Aphanomyces*. At this time blocks of agar containing *Pythium* threads which had grown through the parasitized region where cut out at the places indicated by the two squares on the sides and put on a fresh corn meal agar plate with inoculum of the *Aphanomyces*. See pl. 30, fig. 5.

### PLATE 30

Fig. 5. *Aphanomyces exoparasiticus* and *Pythium* No. 53 inoculated September 14. Examined and figure made Sept. 30. No parasitism. The *Pythium* encroaching upon the *Aphanomyces*.

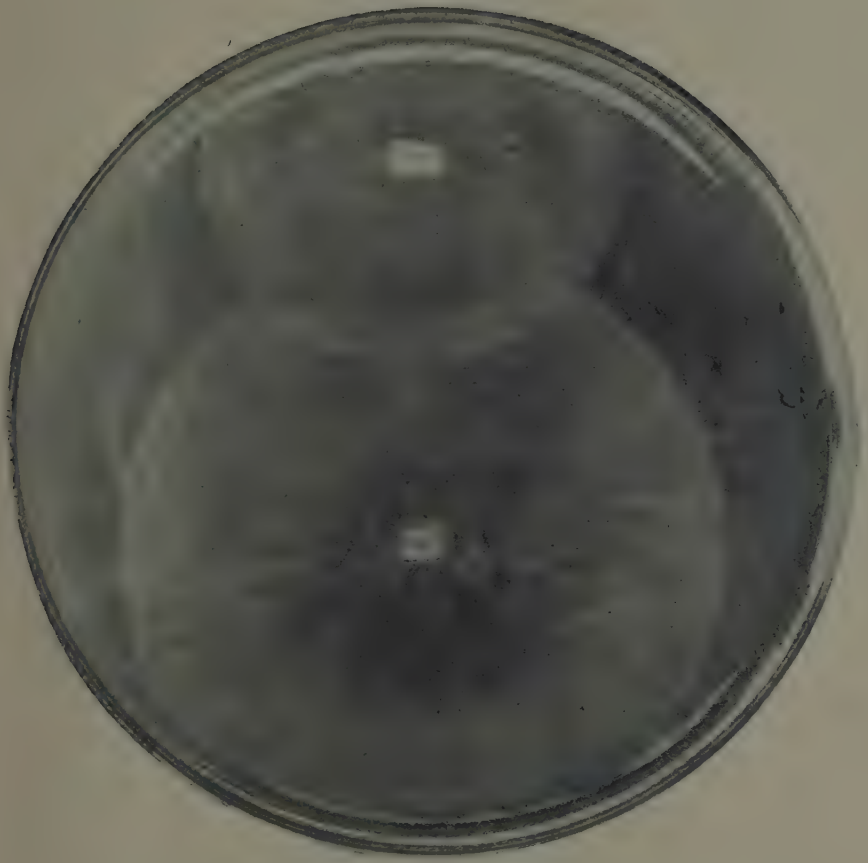
Fig. 6. *Aphanomyces exoparasiticus* above (two inoculations) with *Allomyces arbuscula* below, Sept. 14. In room temperature. Figure made Sept. 30. *Aphanomyces* had covered dish; *Allomyces* had grown very poorly, about 0.4 cm. and was much parasitized.

### PLATE 31

Fig. 7. *Aphanomyces exoparasiticus* with *Allomyces arbuscula* inoculated on Sept. 10 and kept in the oven with the temperature at 34°C. Figure made Sept. 18. The two mats of luxuriant growth below are *Allomyces* while the dwarfed culture to the right above is the *Aphanomyces*. No parasitism.

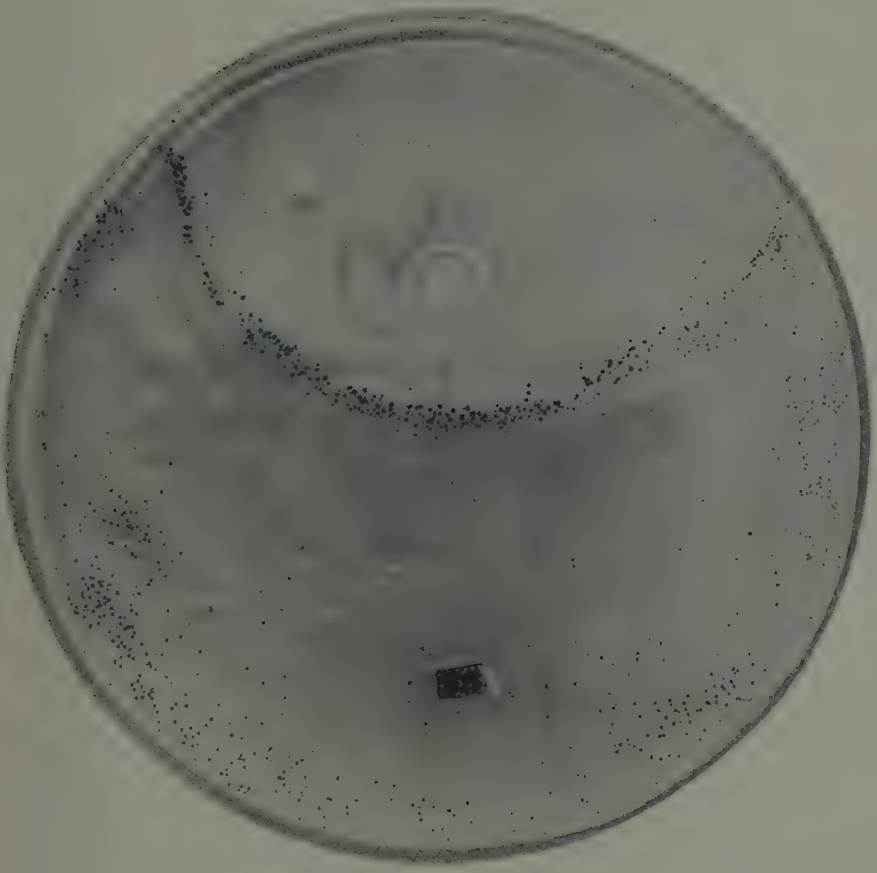
Fig. 8. *Aphanomyces exoparasiticus* above (two inoculations) with *Allomyces arbuscula* below and kept in the oven at 34°C. for two days. Changed to room temperature. Figure made 14 days later. Note the two regions of growth in the *Allomyces:* the inner region formed while the culture was in the oven; the outer, thinner region while out in room temperature.

PLATE 26



*Aphanomyces exoparasiticus* (above) with *Sordaria* sp. (below). × 1¼

PLATE 27



*Aphanomyces exoparasiticus* (above) with *Pythium* sp. (below). ×1½

PLATE 28

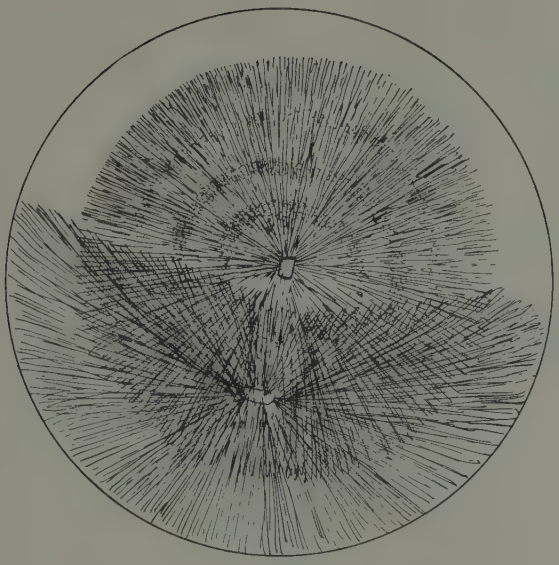


FIG. 1

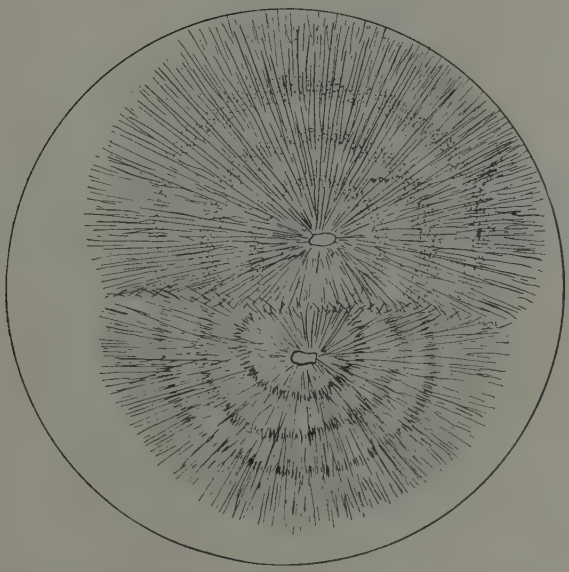


FIG. 2

PLATE 29

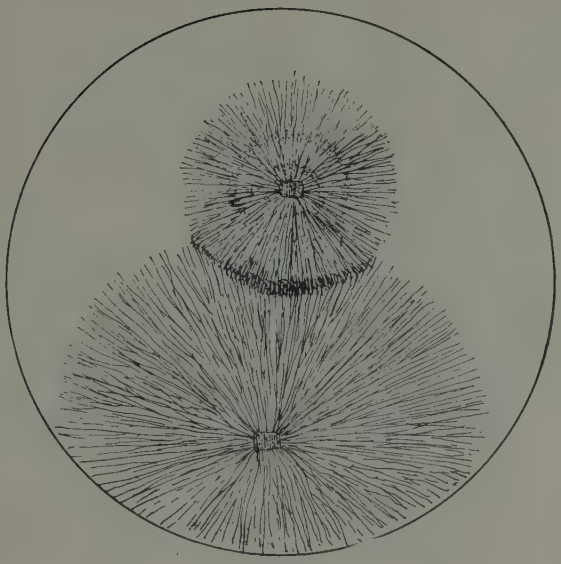


FIG. 3

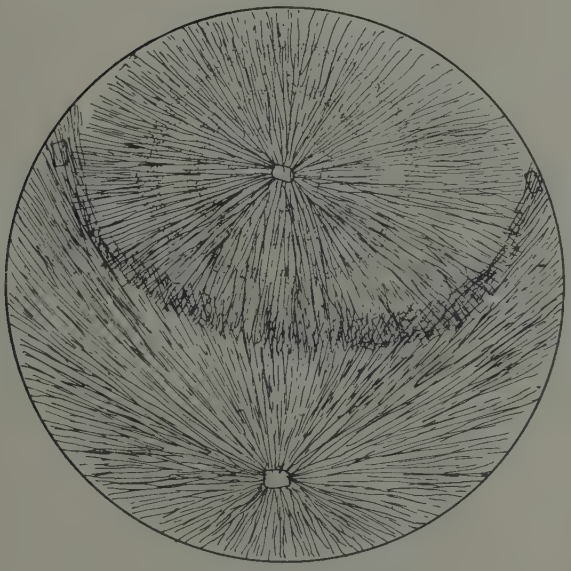


FIG. 4

PLATE 30

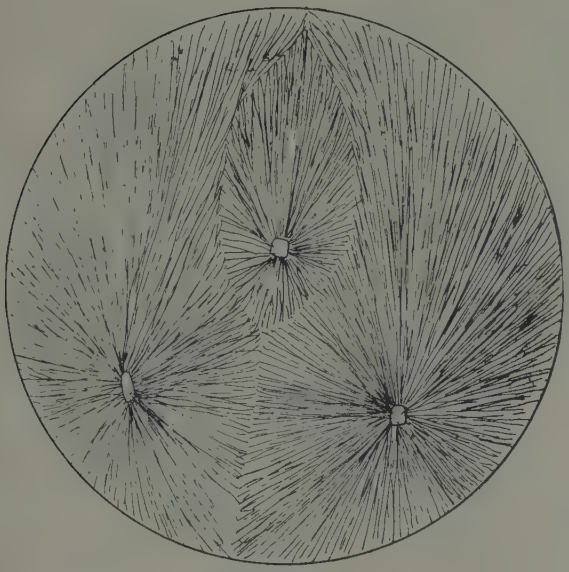


Fig. 5

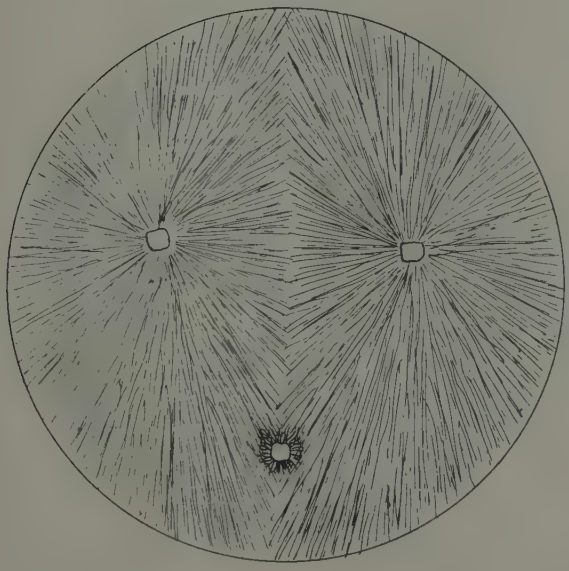


Fig. 6

PLATE 31

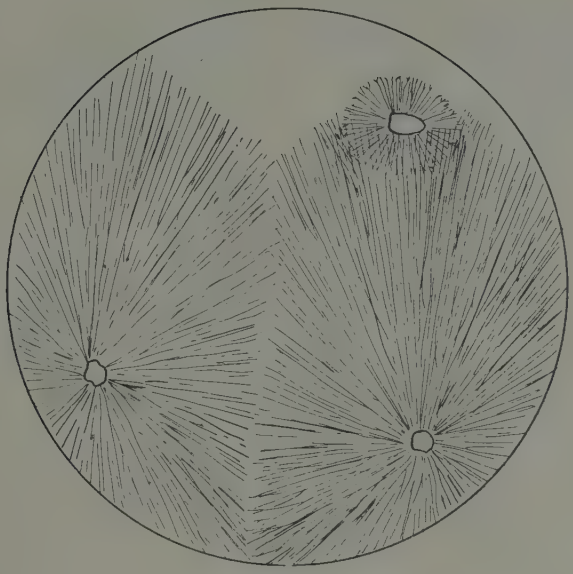


FIG. 7

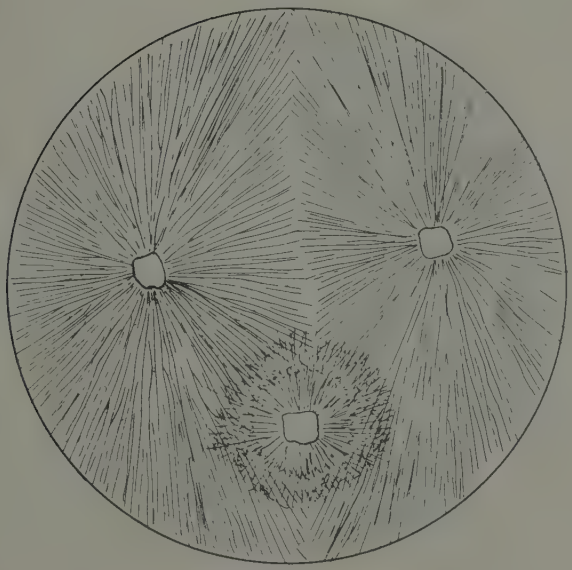


FIG. 8

PLATE 32

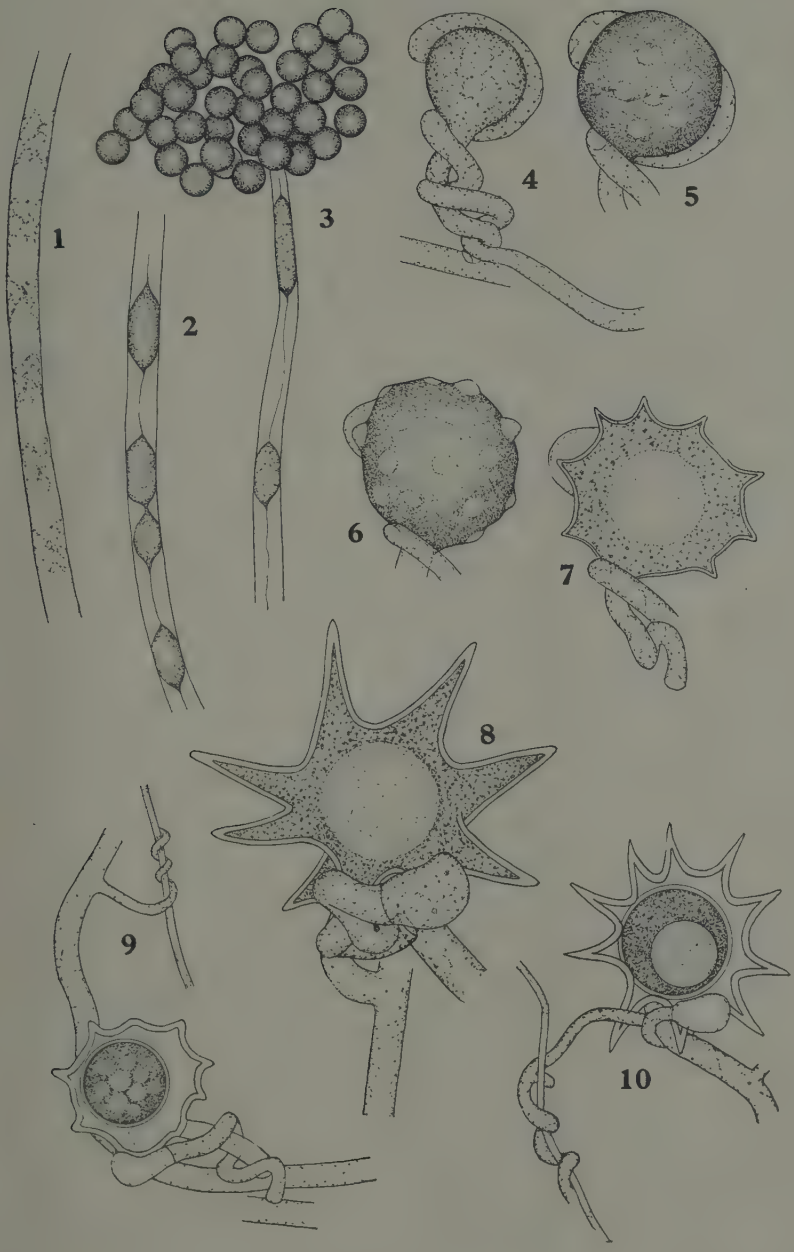
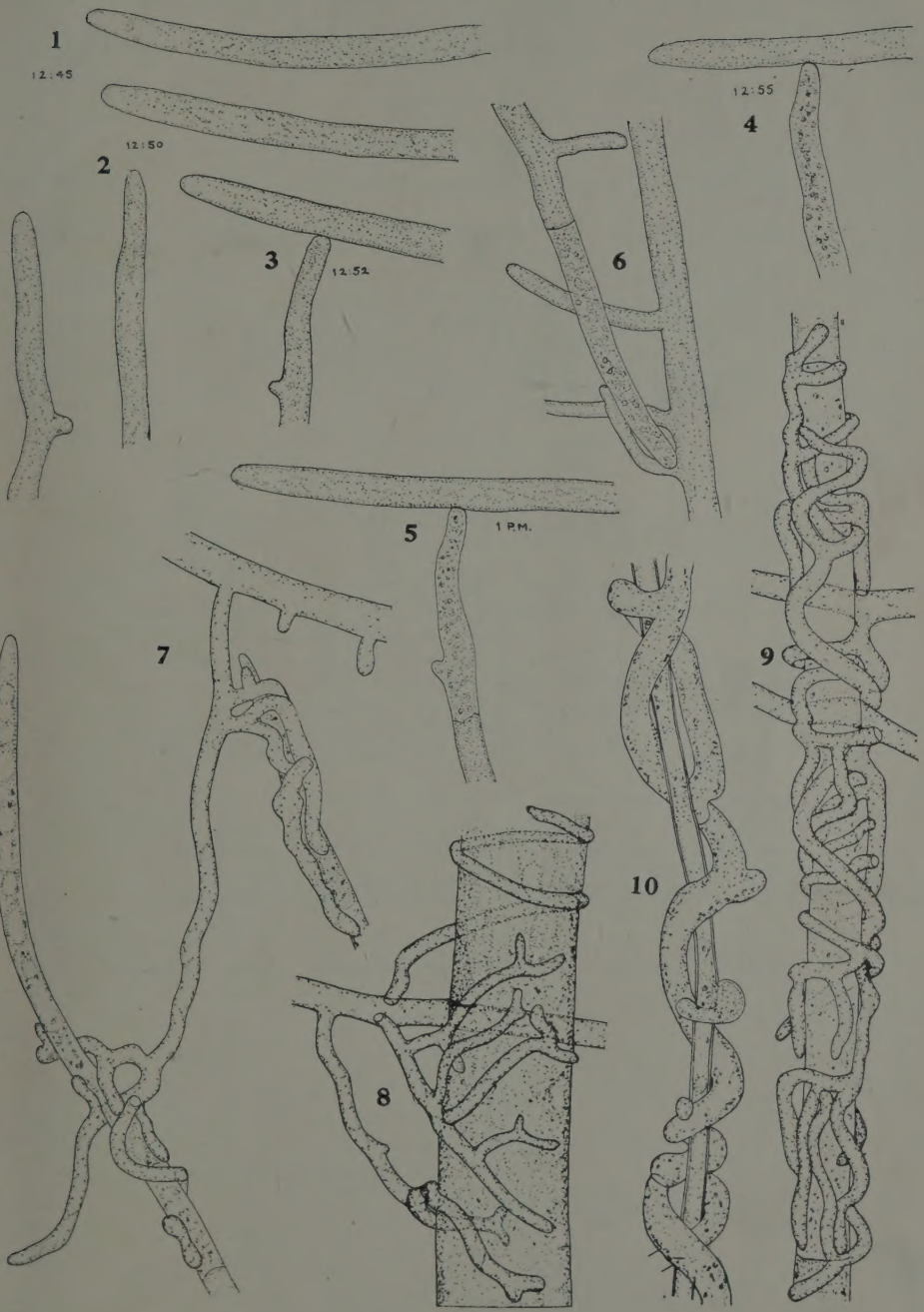


PLATE 33



## PLATE 32

*Aphanomyces exoparasiticus*

Figs. 1, 2, 3. Parts of sporangia showing stages in spore development and emergence. × 502.

Figs. 4–7. Stages in the development of an oogonium. Figures drawn 11 A.M., 2.15 P.M., 5 P.M., and 9 P.M., respectively. × 810.

Fig. 8. Oogonium with characteristically long spines. Twelve spines were visible on this oogonium but only eight are shown in section view. × 1012.

Fig. 9. Oogonium containing a nearly ripe egg. Parasitized *Pythium* thread above. Note empty antheridium and old antheridial tube. × 562.

Fig. 10. Oogonium containing a ripe egg. Antheridial branch arising from a thread coiling around and parasitizing a *Pythium* thread. × 562.

## PLATE 33

*Aphanomyces exoparasiticus*

Figs. 1–5. Early stages in the infection of a *Rhizopus* thread by the *Aphanomyces*. The *Aphanomyces* threads above and horizontal; the *Rhizopus* below and perpendicular. See text. × 413.

Fig. 6. *Aphanomyces* thread to the right sending out a small haustorium around *Rhizopus* thread. Note the cross wall separating the unhealthy region of the *Rhizopus* thread from the healthy. × 413.

Fig. 7. *Aphanomyces* parasitizing the small threads of *Achlya megasperma*. The protoplasmic contents of the parasitized region of the thread in the lower left corner is much vacuolated and coarsely granular; a cross wall separates the unhealthy from the healthy region. × 413.

Fig. 8. A larger thead of *A. megasperma* in early stage of being parasitized. × 413.

Fig. 9. Thread of *A. megasperma* almost completely covered by the *Aphanomyces* haustoria. The *Achlya* thread in this parasitized region seemed to be practically empty of its protoplasmic contents, giving a deep red reaction with congo red while the healthy regions at the ends were very lightly stained. Note the cross walls separating the healthy from the unhealthy region. × 413.

Fig. 10. *Pythium* thread parasitized by the *Aphanomyces*. Note the apparent connection between the host and parasite protoplasm on the right about midway. × 900.